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Acid Hydrolases of the Epidermis: Subcellular Localization and Relationship to Cornification

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Three lysosomal-type acid hydrolases were examined in subcellular fractions of the developing epidermis of fetal rats to assess the relationship of degradative enzymes to cornification. As the granular layer developed and cornified between 18 and 20 days (D) of gestation, epidermal acid phosphatase increased, acid phospholipase A remained constant, and β-glucuronidase activity declined. The enzymes were present in 3,000, 17,000, and 100,000 *g* particulate fractions and soluble cytoplasm. However distribution differed: acid phosphatase and phospholipase A were more preferentially localized than was glucuronidase in the 17,000 *g* fraction which excluded mitochondria and ribosomes and was enriched in lamellar granules. The findings suggested that acid phosphatase and phospholipase were present in membrane-bound organelles (e.g., lamellar granules) in the granular layer. Particulate acid phosphatase increased with granular layers on days 19 and 20 while a 7-fold increase in soluble enzyme coincided with cornification on day 20. As shown by isoelectric focusing, the enzyme became more heterogeneous at day 20 than at day 18, suggesting increased glycosylation. The particulate fraction displayed lysosomal characteristics with respect to release of acid phosphatase, which was inhibited by hydrocortisone and enhanced by retinol. When fetal epidermis was allowed to cornify in organ cultures, similar increases in acid phosphatase occurred. The presence of hydrocortisone did not affect increase in total enzyme but a greater proportion remained in the particulate fraction.

The findings suggest that particulate acid phosphatase and phospholipase are compartmentalized in organelles with lysosomal characteristics during development of granular cells and that release of phosphatase is coincident with cornification. This may reflect not only exocytosis of lamellar granules but also intracellular release of the hydrolytic enzyme.

Cornification of keratinocytes is a complex process which involves, among other things, degradation of many intracellular components. Although the presence of hydrolytic enzymes in epidermis is well established, little is known about their function in the terminal events that occur during formation of cornified cells.

Acid hydrolases, in particular, are associated with intracellular degradative events. They are generally compartmentalized in membrane-limited organelles with specific intra- or extracellular functions (lysosomes). In the epidermis such enzymes include glucuronidases, sulfatases, sphingomyelinase, phospholipases, glycosidases, nonspecific esterases, proteases, and phosphatases [1–4].

Cytochemical studies of epidermis have localized various acid hydrolases to lysosomes, melanosomes, and lamellar granules (LG) (for reviews see [5,7]). It has been shown that acid phosphatase is markedly more prominent in the granular layer [8,9] where it is present in LG, in Golgi apparatus, and related vesicles, and may be visualized diffusely throughout the cytoplasm [10]. It is also present in the intercellular space, after LG are extruded, in both granular and stratum corneum layers. Direct assays of different cell layers suggest that acid phosphatase and pyrophosphatase are increased in granular cells, while the concentration of certain other lysosomal acid hydrolases declines as epidermal cells mature [11]. Moreover, while activation of classic lysosomes has been demonstrated after epidermal insult [12,13], autophagocytosis is not a regular feature of cornification of granular cells [14]. It is thus not yet clear which hydrolytic enzymes play a role in the death of epidermal cells, how these are compartmentalized, or how they are released.

Our prior studies have localized an acid phospholipase A in a post mitochondrial fraction obtained at 17,000 *g* [4]. Subsequently, we have shown that acid phosphatase is also present in this fraction and that both acid phosphatase and acid phospholipase A are associated with LG that have been isolated from the 17,000 *g* fraction [15,16].

In the present studies we attempt to assess the subcellular localization of various acid hydrolases and to correlate such enzymes with ongoing cornification. To this end, we have employed fetal rat epidermis at various stages of development of granular and stratum corneum layers.

MATERIALS AND METHODS

Tissue

Pregnant Sprague-Dawley rats (Holtzman) were obtained with known times and dates of impregnation. Rats were sacrificed at 18, 19, and 20 days of gestation at 9–10 AM with intracardiac Dibutanol. Fetuses were obtained immediately and decapitated. Dorsal and lateral skin was removed and cut into strips. Epidermis was removed after whole skin was immersed for 2 min in 0.22 M NH_4Cl (pH 9.4) at 4°C and stored in cold Earle's buffer until enough tissue was harvested.

Tissue Fractionation

Minced epidermis was homogenized gently in loose-fitting all-glass homogenizer in 0.25 M sucrose at concentrations ranging from 100–200 mg/ml. The crude homogenate was centrifuged for 15 min at 700 *g* and the resulting supernatant was the starting material for further fractionations.

Subcellular fractions were prepared by sequential differential centrifugations at 3,000 *g*, 17,000 *g* and 100,000 *g* for 20, 30, and 60 min, respectively. In organ culture experiments 700 *g* supernatant obtained from epidermal homogenates was directly centrifuged at 17,000 *g* to yield a particulate and supernatant fraction. When pellets were washed

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Abbreviations:
AP: acid phosphatase
APL: acid phospholipase A
BG: β-glucuronidase
D: day(s)
HC: hydrocortisone succinate
LG: lamellar granule(s)
pI: isoelectric point

they were resuspended in 0.25 M sucrose and centrifuged at the original *g* force.

In some experiments, hydrocortisone succinate (Sigma) dissolved in sucrose, or retinol (Sigma) dissolved in ethanol (protected from light), were added to homogenates immediately prior to enzyme assays.

All phases of the preparation were carried out at 4°C.

Organ Cultures of Whole Skin

Skin was obtained from fetuses at 18 days of gestation under sterile conditions. It was cut into 2 × 2 mm squares and explanted dermis side down on nylon cloth rafts supported by steel grids in Falcon plastic organ culture dishes. Culture medium was Minimum Essential Medium (GIBCO) supplemented with 2 mM glutamine, .075% $NaHCO_3$, 10% fetal calf serum, and with 100 units penicillin, 0.25 μg Fungizone, 100 μg streptomycin per ml. Enough medium was added to just cover the 6–7 explants per dish. Humidity was maintained by a ring of water-soaked filter paper around the center well. Cultures were maintained for up to 2 days at 35°C in an atmosphere of 5% CO_2 in air. Epidermis was obtained from cultured explants by NH_4Cl treatment. Epidermis (20–40 mg) from 4 or 5 dishes was pooled for assays; 12–15 dishes were used for each experimental condition.

Enzyme Assays

Acid phosphatase (AP) was assessed in aliquots of homogenates with .06 M *p*-nitrophenyl phosphate (SIGMA) as substrate, .05 M acetate buffer pH 4.8, and .05% Triton X-100 [1]. Assays were conducted with amounts of homogenates equivalent to 0.25–2.5 mg of whole epidermis in a total volume of 0.5 ml at 37°C for 30 min. AP activity was calculated from spectrophotometer readings at 400 nm. The assay was standardized with acid phosphatase of known activity (Sigma).

β-glucuronidase (BG) was assayed in the same way using *p*-nitrophenyl β-glucuronide (Sigma). Since there was less activity of this enzyme, aliquots contained the equivalent of 10–20 mg whole epidermis.

Acid phospholipase A (APL) was assayed as previously described using [2-^{14}C]-linoleate phosphatidyl choline as substrate [4]. Results were expressed as percent or as nmol of substrate hydrolyzed per 45 min incubation period. Cytochrome oxidase was assayed by the method of Wharton and Tzagoloff [17].

Activity of all enzymes was expressed as substrate utilized per assay period per mg protein or μg DNA.

Preliminary studies demonstrated linearity of enzyme activities with time and homogenate concentration. Activities were not affected by NH_4Cl treatment as tested in whole skin. Activity was abolished by heating to 100°C for 10 min.

Proteins were assayed in 700 *g* supernatant or other fractions by the method of Lowry et al [18] and DNA content of whole epidermis by the method of Martin [19].

Isoelectric Focusing

Subcellular fractions were prepared as described above except that epidermis was stored in cold 0.25 M sucrose to avoid contact with electrolytes. Fractions were diluted with equal volumes of 0.1% Triton X-100 in 0.1 M acetate buffer, pH 5.8, for isoelectric focusing. Experiments were carried out on a 110-ml column (LKB) with a gradient of pH 10–3 according to manufacturer's instructions. After electrofocusing for 24 h, 1.0-ml fractions were collected for determination of pH and assay of AP.

Electron Microscopy

Subcellular fractions were monitored by electron microscopy and AP was demonstrated by a modified Gomori technique as previously described [15]. Cultured explants were monitored by light microscopy using 1-μm sections embedded in Spurr-Araldite and stained with toluidine blue.

RESULTS

Morphology of the Subcellular Fractions

3,000 g pellet: Mitochondria were almost entirely recovered in this fraction. The fraction also contained remnants of cornified cell envelopes, aggregates of tonofilaments, clumps of ribosomes, and unidentified amorphous material (Fig 1).

17,000 g pellet: Mitochondria were sparse. Most of the fraction was composed of membranes in vesicular array and of fully developed LG and of membrane-limited organelles, lacking in lamellae, of the same size and shape as LG (Fig 2). Both types of granules contained AP (results not shown, see [15]). Some tonofilaments and amorphous debris were present.

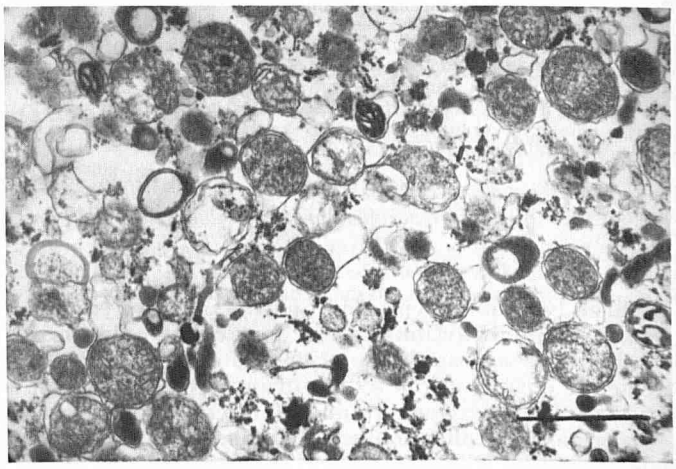

FIG 1. 3000 *g* pellet obtained and processed as per text. Note presence of mitochondria mixed with debris and other organelles. *Bar* = 1 μm.

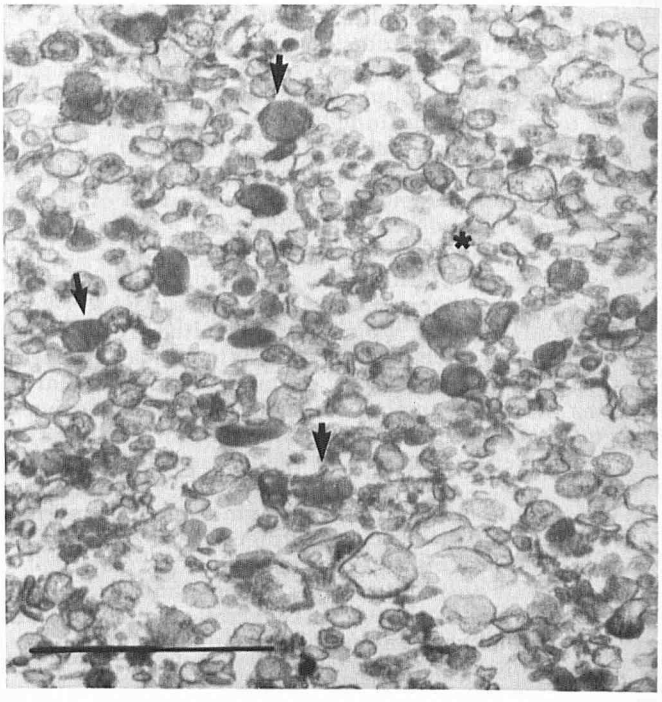

FIG 2. 17,000 *g* pellet obtained as per text. Abundant lamellar granules (*arrowheads*) as well as nonlamellated organelles of the same shape and size (*) containing a granular matrix are present. *Bar* = 1 μm.

This fraction is similar to the lamellar granule fraction previously described [15] but less pure with respect to content of organelles bearing lamellae and contaminating tonofilaments. It was used in preference to the fraction isolated by density gradient fractionation because of the smaller amount of tissue required and more rapid isolation procedures.

100,000 g pellet: This fraction was composed entirely of membranes and ribosomes (results not shown).

Classical lysosomes were seen only rarely in the two large-particle fractions. LG (and LG-like bodies) were present only in the 17,000 *g* pellet.

Enzyme Activity

Acid hydrolases: BG was assessed since this lysosomal enzyme has been shown to be reduced in the granular layer [11].

AP was assessed because of its prominence in granular cells. APL, previously demonstrated in the 17,000 *g* fraction, was further characterized in relationship to the other enzymes. Cytochrome oxidase was assessed as a mitochondrial marker. In epidermis at 20 days (20D) of gestation, 83% of cytochrome oxidase activity was present in the 3000 *g* pellet and 16% in the 17,000 *g* pellet (Table I).

As previously reported, APL was localized in large-particle fractions and soluble cytoplasm [4]. Both BG and AP were distributed in all subcellular fractions (Table I). However, while APL and AP were more abundant in the 17,000 *g* than the 3000 *g* fraction, BG was almost equally distributed in these fractions. Both BG and AP were present in significant quantities in the 100,000 *g* pellet, in contrast to APL.

Specific activities of AP and APL were higher in the 17,000 *g* than 3000 *g* fractions but were not different for BG (Table II). These findings suggested that like APL, particulate AP was preferentially localized in the 17,000 *g* pellet and that BG was not. The presence of significant quantities of all the hydrolases in the soluble cytoplasm cannot be explained at present. In part, it may reflect proteolytic activity and fragility of epidermal membranes with even mild homogenization and separation techniques. However, addition of phenylmethylsulfonyl fluoride, K^+, EDTA, albumin, or serum during preparative stages did not reduce the proportion of enzyme in soluble cytoplasm.

Changes during cornification: Enzymes were assessed at both 18 and 20 days of gestation in 17,000 *g* pellets and supernates. 18D epidermis has no stratum corneum and contains 2 layers of incompletely developed granular cells [20,21], although LG are formed (K Wier and RK Freinkel, unpublished observations). By 19 days there are 5–6 layers of granular cells and the first 1 or 2 layers have cornified. By 20 days, these granular cells have cornified to produce 6–7 layers of squames and have been replaced by 5–6 new layers of granular cells. Since no squames have been shed at this stage, the numbers of cornified layers accurately reflect the numbers of preceding granular cells (K Wier and RK Freinkel, unpublished observations).

When related to wet weight or protein content of the whole epidermis, activities of BG and APL decreased while that of AP increased from 18D to 20D. However, when related to DNA, as an index of nucleated cells, a different picture emerged (Table III). In the 17,000 *g* pellet BG decreased, APL remained unchanged, and AP doubled in activity. Activities of BG and APL were not significantly different in supernates while AP showed a marked increase. The average content of DNA/mg wet weight in 18D epidermis is about twice that at 20D, reflecting the presence of the anucleate stratum corneum in the latter. These results are consistent with a decreased concentration of BG, a constant amount of APL, and an increased amount of AP in the granular layer.

The main increase in total AP occurred rather abruptly in the 24 h between 19 and 20 days (Fig 3). A slight gradual increase in activity of 17,000 *g* pellet occurred over the whole 48-h period and was paralleled by that in 17,000 *g* supernatant from the morning of day 18 to the morning of day 19. By day 20, the activity of the supernatant showed a massive increase representing mostly soluble enzyme (Tables II, III). This latter change parallels the keratinization of the granular layer present at day 19 and emergence of new granular cells.

Characterization of AP: These abrupt changes prompted further investigation of AP.

Heterogeneity of acid phosphatase is well known and has been described in epidermis where 2 or more enzymes of different M_r are present [1,22,23]. Studies were performed to assess

TABLE I. *Distribution of enzymes in subcellular fractions*

Fraction[a]	Percent of total enzyme[b]			
	BG[c]	AP[c]	APL[c]	CYOX[c]
3,000 *g* P	16.4	4.7	15.9	83.0
17,000 *g* P	14.8	7.2	29.1	16.2
100,000 *g* P	17.8	6.8	2.5	[<1.0
100,000 *g* S	49.6	81.2	53.0	

[a] Fractions prepared as per text. P = pellet, S = supernatant.
[b] Results expressed as % of total activity in 700 *g* supernatant in each fraction. Experiment shown is representative of 3 experiments using 20D epidermis.
[c] BG = β-glucuronidase, AP = acid phosphatase, APL = acid phospholipase, CYOX = cytochrome oxidase.

TABLE II. *Specific activities of acid hydrolases in subcellular fractions*

Fraction	Enzyme activity[a]		
	BG	AP	APL
3,000 *g* P[b]	0.5 ± 0.2	4.2 ± 0.9	13.6 ± 1.1
17,000 *g* P	0.4 ± 0.1	6.3 ± 0.9	20.3 ± 1.0
100,000 *g* P	0.3 ± 0.5	3.3 ± 0.3	—[c]
100,000 *g* S	0.2 ± 0.1	9.0 ± 2.4	4.6 ± 0.5

[a] Abbreviations as in Table I. Enzyme activity in various fractions for BG and AP expressed as μmol substrate converted per mg protein ± SEM. For APL, results are expressed as % substrate converted per assay period per 100 μg protein. Results are the mean of 3 experiments using 20D epidermis.
[b] Fractions obtained as for Table I.
[c] Not done.

TABLE III. *Comparative activity of acid hydrolases at 18 and 20 days of gestation*

Fraction	Enzyme Activity × 10^3 per μg DNA[a]		
	BG	AP	APL
18D			
P[b]	1.4 ± .3	2.4 ± .1	3.2 ± 1.1
S[c]	1.2 ± .2	4.6 ± .1	4.4 ± 1.5
Total	2.6	7.0	7.6
20D			
P	0.8 ± .1	5.0 ± .2	2.8 ± 1.6
S	1.0 ± .4	36.4 ± .7	3.3 ± 1.8
Total	1.8	41.4	6.1

[a] Results are expressed as mean ± SEM in 4 experiments using paired litters at 18 and 20 days (D) of gestation. Enzyme activity is expressed as μmol × 10^3 substrate hydrolyzed per μg DNA of whole tissue for β-glucuronidase (BG) and acid phosphatase (AP) and nmol × 10^3 substrate hydrolyzed for acid phospholipase (APL).
[b] P = 17,000 *g* pellet.
[c] S = 17,000 *g* supernatant.

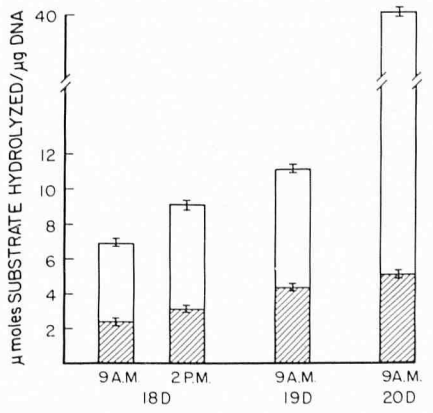

FIG 3. Acid phosphatase at 18 through 20 days of gestation: AP activity assayed as per text in 17,000 *g* pellets and supernatants in epidermis obtained at times indicated. Experiments performed on matched litters: *18D*, 9 AM n = 4; 2 PM n = 2; *19D*, n = 3, *20D* n = 4 where n = number of experiments. Results expressed as substrate hydrolyzed × 10^3 per assay period per μg DNA ± SEM. *Open bar* = supernatant, *shaded bar* = pellet.

whether the "new AP" in 20D epidermis displayed different kinetics and isoelectric points from that of 18D epidermis.

K_m was assessed in 17,000 *g* supernatants and pellets using Lineweaver-Burk plots (Table IV). Although the mean K_m of supernatants was higher than that of pellets, the difference was not statistically significant. Moreover, no difference was noted between the various fractions at 18 and 20 days. Thus, at least in terms of affinity for this substrate, there was no difference in the newly developed enzymes in the 20D epidermis.

Isoelectric focusing revealed 4 distinct peaks in 20D epidermis present in both supernatants and pellets (Fig 4). 18D supernatant contained only the most acid and the most basic peaks. Attempts to definitively resolve activity of 18D pellets were not successful in 3 experiments.

Membrane characteristics of particulate AP: Further attention was focused on the characteristics of the membranes associated with acid hydrolases, particularly of AP.

Release of AP could be demonstrated in washed subcellular fractions incubated at 37°C in 0.25 M sucrose in phosphate-buffered saline (pH 7.4). After incubation homogenates were centrifuged at 17,000 *g* again and enzyme activity assayed in pellet and supernatant. Release was expressed as the percent of total enzyme present in supernatant after incubation. Pellets from 18D epidermis released 5% of total AP in 30 min but pellets from 20D epidermis released 16% (Fig 5). These results suggested that the AP containing particles were "leakier" in the keratinized epidermis or that the association of membranes and enzyme was more tenuous.

When hydrocortisone succinate (HC) was added to 18D washed pellets, it inhibited release of AP in dose-dependent fashion at concentrations from 10^{-5} to 10^{-2} M. HC, however, had only a small effect on release of AP from 20D pellets and this was not statistically significant (Table V).

Conversely retinol increased release of AP from washed 17,000 *g* pellets of 18D epidermis. The vitamin was added in 10 μl of ethanol to experimental pellets and ethanol was added to controls. In a typical experiment 2.9, 3.7, and 5.8% of AP was released from pellets incubated with 0, 10^{-6}, and 10^{-5} M retinol, respectively.

Release of BG and APL from 17,000 *g* pellets was also assessed. No enzyme activity was released into supernatants at either 18 or 20 days (results not shown).

Acid phosphatase in cultured epidermis: 18D skin was cultured as whole skin explants for periods up to 44 h. After 7 h in culture, total epidermal AP had not changed; but after 20 and 44 h, AP increased 2 and 3 times over 0 time values. The amount of supernatant (17,000 *g*) activity increased 3- and 4-fold after 20 and 44 h, respectively. Addition of HC (10^{-3} M) to

TABLE IV. *K_m of epidermal acid phosphatase*

Day of gestation	Subcellular fraction[a]	
	P ± SEM	S ± SEM
18D (n = 6)[b]	6.1×10^{-5}[c] ± 1.7	8.8×10^{-5} ± 0.9
20D (n = 4)	7.6×10^{-5} ± 1.3	8.7×10^{-5} ± 1.8

[a] Subcellular fraction obtained at 17,000 *g*. P = pellet, S = supernatant, ± standard error of the mean (SEM).

[b] n = number of separate experiments.

[c] Experiments were conducted using standard assay with 15–40 μg protein per vessel and substrate concentrations from 0.01–0.1 M·K_m derived from Lineweaver-Burk plots. No statistical difference was present between values for S vs. P or 18D vs. 20D by Student's *t*-test.

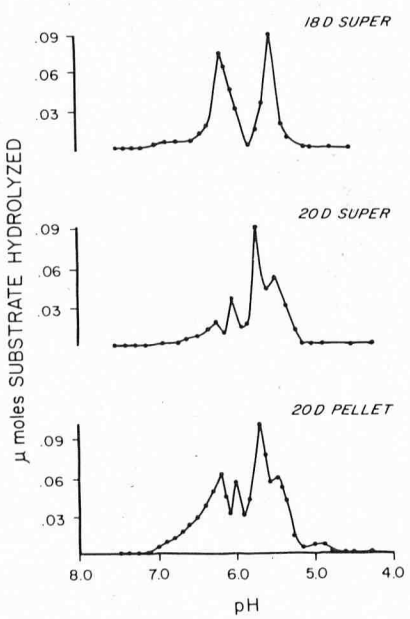

FIG 4. Isoelectric focusing of acid phosphatase. Supernatants and pellets (17,000 *g*) used as per text. The amount of homogenate of the columns was adjusted to approximately 1 mg protein. One-milliliter fractions were collected and pH and acid phosphatase assessed. Results expressed as μmol substrate hydrolyzed per assay period per aliquot. Results are representative of 3 experiments at each time point.

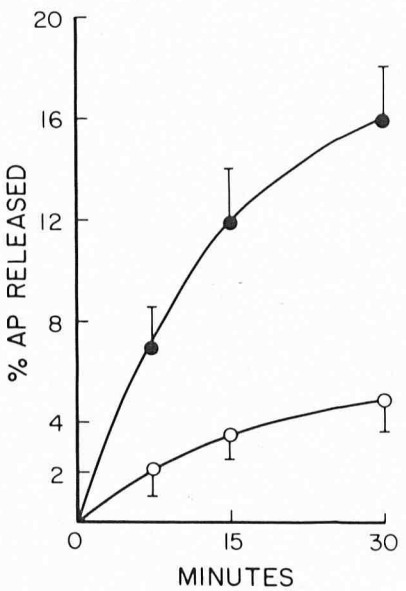

FIG 5. Release of acid phosphatase from particulate fraction. Washed 17,000 *g* pellets of 18D and 20D epidermis were incubated in .25 M sucrose (pH 7.4) for 30 min at 37°C as per text. Release of AP was calculated as percent of total activity present in supernatant. No release of activity could be demonstrated in controls incubated at 4°C. Results shown are the mean ± SEM for 4 experiments at 20 days and 3 experiments at 18 days. ○—○ = 18 day, ●—● = 20 day.

TABLE V. *Effect of hydrocortisone on release of acid phosphatase*

Concentration of HC		Percent control ± SEM[a]	
M/L	(n)[b]	18D	20D
10^{-5}	(4)	88.0 ± 16.9	96.0 ± 8.0
10^{-4}	(6)	83.6 ± 6.6[c]	87.0 ± 7.9
10^{-3}	(4)	67.6 ± 2.8[c]	84.5 ± 9.0
10^{-2}	(2)	57.3 ± 2.5[c]	84.6 ± 6.3

[a] Experiments conducted with washed 17,000 *g* pellets as per text. Released acid phosphatase (AP) assayed in supernatants after incubation at 37°C for 30 min. Results expressed as % of AP released in control vessels incubated without hydrocortisone succinate (HC).

[b] Number of paired experiments at 18 and 20 days (D).

[c] $p < .01$.

TABLE VI. *Acid phosphatase in cultured epidermis*

	Hours in culture[a]	Enzyme activity/mg protein[b]		
		Total	Pellet	Supernatant
A.	0	11.1	4.5	6.6
	7	11.4	4.5	6.9
	20	24.6	4.8	19.8
	44	33.6	6.3	27.3
B.	0	11.7	5.7	6.0
	20 (+ HC)	18.6	7.8	10.8
	20	23.1	4.2	18.9

[a] 18D epidermis was cultured as per text. *A*, Representative of 3 experiments: Epidermis was harvested at designated times from 4 culture dishes. *B*, Representative of 4 experiments: Hydrocortisone succinate, 10^{-3} M, was added to culture medium (+ HC) and epidermis harvested at 20 h from 4 dishes with or without HC.

[b] AP was assessed in 17,000 *g* pellets and supernatants as per text and expressed as μmol substrate hydrolyzed × 10^3 per mg total protein in 700 *g* supernatant.

the culture medium markedly inhibited this increase while particulate activity was increased (Table VI).

Epidermis cultured with or without HC was always increased in thickness, cornified, and showed keratohyaline granules by 20 h. No consistent differences in HC-treated explants with respect to granular layer or cornification could be appreciated by light microscopy (results not shown).

DISCUSSION

In these studies we have focused on the subcellular localization of acid hydrolases during development of granular and stratum corneum layers. A large-particle fraction depleted of mitochondria but enriched in LG was shown to be also enriched in AP and APL but not in BG. Activity of AP increased in this fraction as well as in soluble cytoplasm as the granular layers developed and cornification occurred. This observation suggests that the content of this fraction reflects that of the subcellular organelles of the granular layer. Although keratohyalin is not well developed at 18 days, the most differentiated cells of this epidermis do contain and extrude LG, form marginal bands, and condense albeit without a keratin pattern [21].

While AP increased in the particulate fraction, BG declined, suggesting that this lysosomal enzyme is not associated with the granular cell or cornification. On the other hand, APL which is most specifically localized in the particulate fraction did not change in relationship to cell numbers as judged by DNA. These findings could be due to an increasing accumulation of AP within organelles containing a constant quantity of APL (e.g., LG) in addition to a more homogeneous distribution of APL among the other membranes represented in the 17,000 *g* fraction.

The presence of acid hydrolases in 17,000 *g* supernatants deserves some additional comment. Some portion is attributable to enzymes in membranes and ribosomes (Table II), but the majority is in soluble cytoplasm. It is likely that some of this apparent distribution in cell cytoplasm results from artifacts attending subcellular fractionation. Indeed this has been demonstrated previously for APL [4].

Nonetheless, the increase in AP, coincident with cornification, appears to reflect a real increase in soluble enzyme. Much of this may be attributed to the presence of AP in the intercellular spaces of stratum corneum where it is probably no longer bound to or limited by membranes. In this site, the AP must derive from the extruded contents of LG. However, it is also probable that AP is released from membranes within the granular cell as it begins to cornify. Whether it is sequestered initially in some as yet not defined organelles or directly released after synthesis remains to be ascertained. In either event the presence of so much soluble AP may subserve some specific function at this stage of the life of the cell. It has been suggested that it participates in the degradation of nucleotides, phospholipids, and phosphorylated carbohydrates as well as in the regulation of pyridoxal phosphate [23,24]. Another possibility is that AP is concerned with dephosphorylation of the precursor of stratum corneum basic protein prior to its aggregation with keratin [25].

In this context we attempted to determine whether there were qualitative differences between the AP found at 18 and 20 days of gestation in soluble and particulate fractions. Our studies have confirmed the heterogeneity that others have described for epidermal AP [1,22,23,26] and also have shown that increased complexity of AP accompanies cornification. Mäkinen and Mäkinen have recently identified 3 isoenzymes of AP in rat epidermis [23]. The 2 major ones have isoelectric points (pI = 5.3–5.5 and 5.5–6.2) similar to the 2 present in 18D epidermal supernatants (pI = 5.5 and 6.2). A very high M_r AP with pI = 4.3 reported by those authors was not demonstrated in our experiments; this AP was solubilized after lengthy treatment with Triton X-100 and may thus have been missed in our experiments. On the basis of different sensitivity to inhibition by tartrate and fluoride or formalin as well as labilization by detergents, Mäkinen and Mäkinen concluded that the high M_r AP (pI = 5.3–5.5) was particulate and lysosomal while the low M_r AP (pI = 5.5–6.2) was cytoplasmic. However, our findings are more in agreement with those of Okhawara et al [1] who demonstrated the 2 similar molecular species in 50,000 *g* pellets and supernatants in human epidermis. In 20D rat epidermis the 4 isoenzymes demonstrated by isoelectric focusing were qualitatively similar in both pellets and supernatants.

Heterogeneity of AP in rat epidermis [23] and psoriatic scale [26] has been also demonstrated to arise from varying degrees of glycoslylation with n-acyl neuraminic acid. The greater complexity of enzymes in cornified 20D epidermis vs. that of 18D epidermis may thus reflect emergence of different molecular species and/or different degrees of glycosylation of the same species. Resolution of this question will require additional studies on more purified preparations.

Although function of AP in LG defies speculation, APL may play a role in membrane perturbations attending fusion of LG with plasma membranes during exocytosis [27]. It could also play a role in the putative degradation of LG phospholipids and thus help to align the lipids that participate in the barrier function of the intercellular space [28].

The characteristics of membrane association of AP were of particular interest. Release of enzyme with incubation, stabilization of membrane association by HC, and labilization by retinol are characteristic of lysosomal type organelles. The implication of the present data is that the membranes of LG share these characteristics, although this inference must await confirmation with purer preparations of LG. The enhanced lability of AP in keratinized tissue may reflect the labile status of mature LG in the process of exocytosis. The failure of HC to affect this supports this interpretation.

Finally the effect of HC in cultured epidermis has interesting implications. AP increased in both soluble and particulate fractions when 18 day epidermis keratinized in organ cultures. The changes were similar to those occurring in vivo. Although the presence of HC did not significantly retard the increase in total AP, it did reduce the proportion of soluble enzyme while increasing the amount present in the particulate fraction. Thus HC appeared to stabilize LG (and other membranes) in the whole-cell preparation. If such an effect results in impeded exocytosis of LG and of the hydrolytic effects of AP, it may afford a basis for the effects of HC in keratinization deserving further exploration.

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REFERENCES

1. Ohkawara A, Halprin KM, Taylor JR, Levine V: Acid hydrolases in human epidermis. Br J Dermatol 83:450–459, 1972

2. Mier PD, van den Hurk JJMA: Lysosomal hydrolases of epidermis. Br J Dermatol 93:1–10, 391–398, 509–517, 1975
3. Bowser PA, Gray GM: Sphingomyelinase in pig and human epidermis. J Invest Dermatol 70:331–335, 1978
4. Freinkel RK, Traczyk TN: The phospholipases A of epidermis. J Invest Dermatol 74:169–173, 1980
5. Rowden G: Ultrastructural studies of keratinized epithelia of the mouse. J Invest Dermatol 49:181–197, 1967
6. Hori Y, Toda K, Pathak MA, Clark WH Jr, Fitzpatrick TB: A fine structural study of the human epidermal melanosome complex and its acid phosphatase activity. J Ultrastruct Res 25:109–120, 1968
7. Wolff-Schreiner EC: The ultrastructural cytochemistry of the epidermis (review). Int J Dermatol 16:77–102, 1977
8. Moretti G, Mescon H: Histochemical distribution of acid phosphatase in normal skin. J Invest Dermatol 26:347–360, 1956
9. Hashimoto K, Ogawa K: Histochemical studies in skin. 1. The activity of acid phosphatase during histogenesis of the skin of the rat. Am J Anat 113:35–50, 1963
10. Eisen AZ, Arndt KA, Clark WA: The ultrastructural localization of acid phosphatase in human epidermis. J Invest Dermatol 43:319–326, 1964
11. Mier PD, Cotton PWK, van den Hurk JJMA, Jonckheer-Vanneste MMH: Lysosomal hydrolases of the epidermis, 5: Variation with depth in the cow snout. Br J Dermatol 94:535–538, 1976
12. Johnson BE, Daniels F: Lysosomes and reactions of the skin to ultraviolet radiation. J Invest Dermatol 53:85–93, 1969
13. Hönigsmann H, Wolff K, Konrad K: Epidermal lysosomes and ultraviolet light. J Invest Dermatol 63:337–342, 1974
14. Wolff K, Schreiner E: Epidermal lysosomes. Arch Dermatol 101:267–286, 1970
15. Freinkel RK, Traczyk TN: Isolation and partial purification of lamellar granules from fetal rat epidermis. J Invest Dermatol 77:478–482, 1981
16. Freinkel RK: The lipids and acid hydrolases of lamellar granules. Clin Res 30:584A, 1982
17. Wharton DC, Tzagoloff A: Cytochrome oxidase from beef heart mitochondria, Methods in Enzymology, vol 10. Edited by RW Estabrook, ME Pullman. New York, Academic, 1966, pp 245–250
18. Lowry OH, Rosenbrough WJ, Farr AL, Randall RT: Protein measurement with Folin reagent. J Biol Chem 193:265–275, 1951
19. Martin RF: Estimation of DNA in tissue. Proc Australian Biochem Soc 3:89, 1970
20. Bauer FW: Differentiation and keratinization of fetal rat skin. Dermatologica 145:16–36, 1972
21. Buxman MM, Kocarnick MJ, Holbrook KA: Differentiation markers in fetal epidermis: transglutaminase and transpeptidase. J Invest Dermatol 72:171–177, 1979
22. Miyagawa T, Koda H, Urabe H: Chromatographic fractionation and partial characterization of acid phosphatase in guinea pig epidermis. J Invest Dermatol 63:476–478, 1974
23. Mäkinen PL, Mäkinen KK: Purification and properties of rat skin acid phosphatases. Int J Pept Protein Res 18:352–369, 1981
24. Miyagawa T, Anai M, Urabe H: Degradation of deoxyribonucleic acid by guinea pig epidermal extracts. Arch fur Dermatologische Forsch 254:79–81, 1975
25. Dale BA, Lonsdale-Eccles JD, Holbrook KA: Stratum corneum basic protein: an interfilamentous matrix protein of epidermal keratin. Current Problems in Dermatology 10. Edited by IA Bernstein, M Seiji. Basel, S Karger, 1980, pp 311–325
26. Miyagawa T, Doi F: Multiple forms and glycoprotein nature of acid phosphatase, α-fucosidase, and α-mannosidase of psoriatic scales. J Invest Dermatol 73:554–557, 1979
27. Poole AR, Howell JI, Lucy JA: Lysolecithin and cell fusion. Nature 227:810–814, 1970
28. Elias PM, Goerke J, Friend DS: Mammalian epidermal barrier lipids: composition and influence on structure and function. J Invest Dermatol 69:535–546, 1977

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Distribution of Fibronectin and Laminin in Basal Cell Epitheliomas*

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The distribution of fibronectin (FN) and laminin (LM) in basal cell epithelioma was evaluated by indirect immunofluorescence. FN is a glycoprotein which promotes interaction between cells and the extracellular matrix, and is present at the dermal-epidermal junction (DEJ) and throughout the dermis, but absent in the normal epidermis. LM, a noncollagenous basement membrane protein, plays a role in epithelial adhesion to type IV collagen, and is normally present in the DEJ, but absent in the epidermis. The role of FN and LM in epithelial differentiation has not been established. Therefore, the distribution of FN and LM in a tumor of epithelial origin was studied by indirect immunofluorescence. Using affinity-purified antibodies to FN and LM, and the appropriate fluorescein-conjugated second antibodies, normal skin and 7 basal cell tumors were examined. By immunofluorescence, nests of malignant basal cells were surrounded by linear LM staining. FN immunofluorescence was intense throughout the connective tissue stroma of all tumors. Five tumors also showed FN staining within the nests of neoplastic cells, and 4 of these were also LM-positive in the tumor bulk. These immunofluorescent findings suggest that epidermal neoplasia can be associated with alterations in the distribution of FN and LM.

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Abbreviations:
DEJ: dermal-epidermal junction
FN: fibronectin
LM: laminin
PBS: phosphate-buffered saline

Fibronectin (FN) and laminin (LM) are major proteins of the connective tissue matrix. FN is found in extracellular matrices where it is frequently associated with the cell surface. In transformed cells, synthesis of FN is significantly diminished, and although secretion of the protein is not affected, little or no FN